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# Physicochemical Factors Affecting the Uptake by Roots and Translocation to Shoots of Amine Bases in Barley

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**Abstract:** The uptake by barley roots from nutrient solution and subsequent transport to shoots of two series of amine bases were measured over 6 to 72 h. The compounds were chosen to span systematically ranges of lipophilicity (assessed using 1-octanol/water partition coefficients, $K_{ow}$) and pKa that would include commercial pesticide amines. In a series of six substituted phenethylamines, strong bases with pKa ~ 9·5, all the compounds were strongly taken up by roots from solutions of pH 8·0; uptake declined substantially as the pH was lowered to 5·0, especially for the compounds of intermediate lipophilicity (log $K_{ow}$ 2 to 3). This uptake could be ascribed to three processes: (i) accumulation of the cation inside the root cells due to the negative charge on the plasmalemma, as given by the Nernst equation and important only for the polar compounds which have low permeation rates through membranes; (ii) accumulation into the vacuole by ion-trapping, which was the dominant process at high pH for all compounds and at all pH values for the compounds of intermediate lipophilicity; (iii) partitioning on to the root solids, substantial only for the most lipophilic compounds. Translocation to shoots was proportional to uptake by roots, this ratio being independent of external pH for each compound and being optimal for the compounds of intermediate lipophilicity. Such proportionality was also observed in a series of three weaker bases of intermediate lipophilicity, in which compounds of pKa 7·4 to 8·0 were also well taken up and translocated whereas the very weak base 4-ethylaniline (pKa 5·03) was much less so. Tests with quaternised pyridines confirmed that organic cations move only slowly through membranes. The observed behaviour of the amines could be modelled reasonably well assuming that transport within the plant was dominated by movement across membranes of the non-ionised species, and this appeared to be true even for the most lipophilic phenethylamine (log $K_{ow}$ 4·67) studied, though its long-distance movement would be as the protonated species. © 1998 Society of Chemical Industry

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Key words: root uptake; translocation; amines; *Hordeum vulgare;* log $K_{ow}$; pH; pKa

## 1 INTRODUCTION

The uptake and transport of pesticides in plants are important both for the activity of many such compounds (i.e. systemic pesticides) and also for understanding the likely distribution of pesticide residues in crop plants at harvest. Bromilow and Chamberlain[1] have reviewed such systemicity and its relationships to the physicochemical properties of pesticides.

Long-distance transport can be either *via* the xylem or phloem vascular systems. Non-ionised compounds are translocated primarily *via* the xylem, i.e. with the transpiration stream in the direction from the roots to the shoots, though some of the more polar compounds may be also weakly moved in phloem, i.e. with the photosynthesate stream from leaves to the growing points in shoots and roots. Such transport patterns

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have been explored in terms of the permeation rates of non-ionised molecules through membranes; this process is a function of the lipophilicity of the pesticide, with compounds of intermediate lipophilicity being best able to cross plant membranes.[2–4]

The transport of weak acids has also been thoroughly studied, as many herbicides have this functionality and rely on being transported in the phloem to reach their site of action. Again movement across plant membranes controls the transport pattern, but now the situation is more complex insomuch as at least two species, the undissociated acid and its corresponding anion for monobasic acids, are present and these have very different permeation characteristics. Furthermore, the proportions of these species will differ according to the pH of the plant compartment; since the undissociated form generally crosses membranes much more rapidly than its anion, weak acids tend to be accumulated in the plant compartments of higher pH such as the cytoplasm and phloem.[5,6] This process is called ion trapping, and can be estimated from the Nernst equation;[6,7] this requires knowledge of the pH values of the plant compartments and any external solution, the Donnan charge on the membranes bounding the compartments and the relative permeation rates of the undissociated acid and its anion.

Systemicity of bases has been much less studied. Some herbicides, such as paraquat, are quaternised pyridine bases and are taken up and moved in plants, but such transport is very limited due to their rapid phytotoxicity in the light.[8] Price *et al.*[9] observed that, following application to wheat leaves, 1-methylpyridinium chloride and related quaternised pyridine salts were translocated both in xylem and in phloem. Hsu *et al.*[10] studied the phloem transport of a series of quaternised amines, and related this to their lipophilicities. Several fungicides are amine bases, either weak bases such as carbendazim (pKa 4·2) or stronger bases such as tridemorph (pKa 7·4) and dodine (pKa $\sim$ 12). These compounds were amongst the first systemic fungicides and were introduced in the late 1960s. Tridemorph is an example of the so-called morpholine fungicides whose systemic properties are slightly surprising given the high lipophilicity of these compounds; clearly the more polar protonated species must be implicated in such transport, for non-ionised molecules of such high lipophilicity are not systemic.[2] Furthermore, it has recently been shown that dodemorph and tridemorph are taken up by the roots of barley plants from nutrient solutions and translocated to the shoots with remarkable efficiency, such uptake being dependent on the nutrient solution pH and being most effective at higher pH.[11]

The studies reported here examined systematically the role of lipophilicity in influencing the uptake and transport in barley of a series of strong phenethylamine bases, together with examining the role of pKa using a set of bases of approximately constant and intermediate lipophilicity. Further tests have been done using two quaternised amines to investigate independently the permeation rates of organic cations through plant membranes. The processes involved in the systemicity of these bases are considered in relation to their physicochemical properties, such that predictions may be made for other basic compounds.

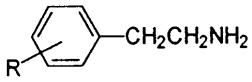
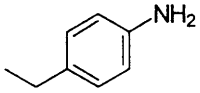
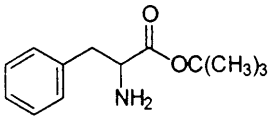
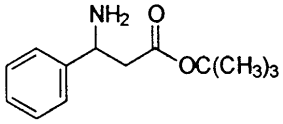
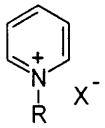
## 2 MATERIALS AND METHODS

### 2.1 Compounds

The structures and physicochemical properties of the compounds studied are given in Table 1. The syntheses of some are described below; others were purchased from commercial suppliers. All compounds were chromatographically pure. NMR spectra for the synthesised compounds were consistent with their structure.

*2.1.1 Synthesis of substituted phenethylamines (Series I)*
3-Phenoxyphenethylamine and 3-(3,4-dichlorophenoxy)-phenethylamine were synthesised from the substituted benzaldehydes. Lithium aluminium hydride (15 mmol) was dissolved in anhydrous diethyl ether (36 ml) and the appropriate benzaldehyde (55 mmol) dissolved in anhydrous ether (9 ml) was then added. The mixture was stirred under reflux for 30 min, cooled and small amounts of water added until generation of hydrogen stopped, and then acidified with 10% sulfuric acid. The ether was separated, washed with water, dried over sodium sulfate and evaporated to leave the crude benzyl alcohol which was recrystallised from benzene. Thionyl chloride (250 mmol) was added to the benzyl alcohol (25 mmol) and the mixture stirred at room temperature for 24 h and then evaporated to leave the benzyl chloride. This was converted to the phenylacetonitrile by refluxing the appropriate benzyl chloride (23 mmol) and sodium cyanide (28 mmol) in ethanol (250 ml) and water (15 ml) for 7 h. The cooled reaction mixture was evaporated and the product extracted into ether which was then dried over sodium sulfate and evaporated to leave the nitrile. To reduce the nitrile to the amine, sulfuric acid (*d* 1·84; 18 mmol) was added to lithium aluminium hydride (37 mmol) in anhydrous ether (22 ml) cooled in an ice bath, and then stirred at room temperature for 1 h. The phenylacetonitrile (11 mmol) in anhydrous ether (9 ml) was added dropwise with vigorous stirring and then stirred under reflux for 2 h. Small amounts of water were added to the cooled reaction mixture until hydrogen was no longer evolved. Sodium hydroxide solution was added and the mixture filtered. The aqueous layer was discarded and the ether partitioned with 0·5 M hydrochloric acid. The aqueous phase was separated, adjusted to pH $>$ 12 and extracted with dichloromethane, which extract was then evaporated to

**TABLE 1**
Structures of Bases, with Log $K_{ow}$ and pKa Values

| *Base* | *Log* $K_{ow}$ (*free base*) | *pKa* |
|---|---|---|
| *Series I* (*similar pKa, various* $K_{ow}$)<br>Substituted phenethylamines | | |
|  | | |
| R = 4-mesyl | −0·04 | 9·54 |
| 4-$NO_2$ | 1·26 | 9·48 |
| 4-F | 1·63 | 9·83 |
| 4-Cl | 2.16 | 9·80 |
| 3-phenoxy | 3·30 | 9·31 |
| 3-(3,4-dichlorophenoxy) | 4·67 | 9·28 |
| *Series II* (*similar log* $K_{ow}$, *various pKa*)<br>4-ethylaniline | 1·95 | 5·03 |
|  | | |
| L-phenylalanine, *tert*-butyl ester | 2·50 | 7·44 |
|  | | |
| *tert*-butyl 3-amino-3-phenylpropanoate | 2·33 | 8·03 |
|  | | |
| *Series III* (*quaternary pyridinium salts*) | | |
|  | | |
| R = n-hexyl X = Br | −1·98 | — |
| n-dodecyl Cl | 0·18 | — |

leave the phenethylamine. For convenience, this was converted to hydrochloride by stirring the substituted phenethylamine (4 mmol) with concentrated hydrochloric acid (6 mmol) and filtering off the precipitated solid which was washed with ethanol and ether. Overall yields were 5 ~ 10%.

4-Mesylphenethylamine was synthesised from 4-(methylthio)phenethylamine. The latter compound was first acetylated by dissolving 6 mmol in pyridine (1·5 ml), cooling and adding acetyl chloride (12 mmol) dropwise with vigorous stirring. The product was obtained by diluting with ice water (10 ml) and filtering off the solid. It was air dried, dissolved in acetic acid (7 ml) and stirred while adding hydrogen peroxide (30%, 3 ml). After stirring at room temperature for 24 h, the solution was poured into water (10 ml) and then evaporated to dryness under reduced pressure to leave a colourless oil from which *N*-acetyl-4-mesylphenethylamine was obtained by chromatography on a column of silica with hexane + acetone (3 + 7 by volume) as eluent. This compound (3·8 mmol) was hydrolysed by heating under reflux with sodium hydroxide solution (4 M, 5 ml) for 1 h. The cooled solution was extracted with ether (3 × 5 ml) and the combined extracts then extracted with small amounts (2 ml) of hydrochloric acid (0·5 M) until they ceased to give any cloudiness on basification. The combined acid extracts were made alkaline and the product extracted with ether (3 × 5 ml)

which was dried over sodium sulfate and evaporated to leave a pale yellow oil which rapidly crystallised. Overall yield was 30%.

*2.1.2 Synthesis of other bases (Series II)*

The *tert*-butyl ester of 3-amino-3-phenylpropanoic acid was prepared from the *N*-benzyloxycarbonyl-protected amino acid by dissolving 17 mmol in dichloromethane (250 ml) and bubbling isobutylene (36 g) into the cooled solution. Concentrated sulfuric acid (1 ml) was added and the flask sealed with a rubber septum. The mixture was stirred overnight at room temperature. The pressure was released with a syringe needle and the solution washed with saturated sodium hydrogen carbonate solution (3 × 50 ml). The organic layer was evaporated to leave an oil which solidified. This was dissolved in a solution of formic acid in methanol (4·4% w/v, 95 ml) and added under nitrogen to a suspension of palladium black (1·9 g) in the same mixture (95 ml). After 5 min at room temperature, the catalyst was filtered off and the filtrate diluted with water (200 ml). Most of the methanol was removed under reduced pressure and the aqueous solution neutralised with sodium hydrogen carbonate. The product was extracted with ether, which was washed, dried and evaporated to leave a yellow oil. Overall yield was 45%.

*2.1.3 Synthesis of substituted pyridinium salts (Series III)*

*N*-Hexylpyridinium bromide was prepared by heating equimolar quantities of 1-bromohexane and pyridine under nitrogen at 120°C for 24 h. A little dry ether was added to the cooled mixture and crystals started to form. After 1–2 h these were filtered off and washed with dry ether. The yield of extremely hygroscopic product was 67%.

## 2.2 1-Octanol/water partition coefficients ($K_{ow}$) and acid dissociation constants (pKa)

Most log $K_{ow}$ values and all pKa values were obtained by a pH-metric method, using a PCA 101 (Sirius Analytical Instruments Limited) automated titrator which incorporated a microcontroller and dedicated computer.[12] The pKa was measured potentiometrically by acid titration in aqueous solution. An appropriate amount of 1-octanol was then added to the aqueous solution and the compound allowed to partition between the two phases. The resulting shift in pKa is related to the log $K_{ow}$ of the compound, and values for both were obtained by computation using non-linear least-squares procedures.

Values of log $K_{ow}$ for the most lipophilic and polar compounds (3-phenoxyphenethylamine, 3-(3,4-dichlorophenoxy)phenethylamine and 4-mesylphenethylamine) were obtained by a standard shake-flask procedure, using 0·1 M sodium hydroxide for the partitioning of the undissociated form of the base. Concentrations in both octanol and water were measured by high-pressure liquid chromatography (HPLC). The value for *N*-hexylpyridinium bromide was taken from the literature and that for the *N*-dodecyl analogue was measured at neutral pH by shake-flask methods as above.[10]

## 2.3 Growth and treatment of barley plants

Details of the experimental procedure have been published,[2] and only an outline is given here together with the slight modifications made for these tests. Seeds of barley (*Hordeum vulgare* L. cv. Alexis), germinated on moist tissue paper, were transferred to aerated nutrient solution (half-strength Hoaglands), and grown in a controlled environment with a 16 h day (10 klux) at 20°C and 8 h night at 16°C.

To measure the uptake of the chemical under test, groups of six 10-day-old plants were transferred to the buffer solution (100 ml) containing the chemical at an initial concentration of 50 μM. Transpiration was measured by weighing the vessel and plants at the beginning and end of each test, when the concentration of chemical in the solution was also measured. Uptake of the compounds of Series I was measured at four pH values from 5·0 to 8·0, with two replicates (each of six plants) at each pH. The behaviour of chemicals of Series II and III was investigated similarly but at five pH values from 4·0 to 8·0. Solutions were buffered with 0·01 M potassium dihydrogen orthophosphate (pH 4·0 to 7·0) and 0·01 M TRIS [2-hydroxy-1,1-bis(hydroxymethyl)ethyl ammonium chloride] for pH 8·0. pH values were checked during the tests after 6 and 24 h and adjusted back to the original pH if necessary by addition of a little 0·5 M sodium hydroxide or hydrochloric acid. This procedure gave reasonable pH control within ±0·3 pH units except for pH 4 where drift to pH ~ 4·6 occurred. At pH 8·0, some of the chemicals were strongly taken up by the roots so that the concentration in the test solution fell markedly, and in these tests the plants were transferred after 2 h to a fresh solution at the original concentration.

## 2.4 Measurement of chemicals

*2.4.1 Extraction and measurement of compounds in plants*

The combined plant replicates (roots or shoots) were homogenised with a freshly prepared 9 + 1 (by volume) mixture of acetone and aqueous 0·1 M hydrochloric acid (total 100 ml in 30- to 40-ml portions), and the extracts were filtered through glass wool into a round-bottomed flask and then evaporated at a bath temperature not

**TABLE 2**
HPLC Conditions for the Amine Bases

| *Compound* | *Organic solvent* | *Buffer* | *pH of buffer* | *Ratio of organic solvent/ buffer (v/v)* | *Wavelength (nm)* | *Retention time (min : s)* |
|---|---|---|---|---|---|---|
| 4-Mesylphenethylamine | $CH_3CN$ | 10 mM phosphate | 8 | 7/3 | 225 | 4 : 13 |
| 4-Nitrophenethylamine | $CH_3OH$ | 10 mM phosphate + 5 mM hexane-sulfonic acid | 3 | 4/6 | 275 | 5 : 55 |
| 4-Fluorophenethylamine | $CH_3CN$ | 10 mM phosphate | 8 | 7/3 | 210 | 13 : 12 |
| 4-Chlorophenethylamine | $CH_3OH$ | 10 mM phosphate + 5 mM hexane-sulfonic acid | 3 | 1/1 | 220 | 5 : 52 |
| 3-Phenoxyphenethylamine | $CH_3OH$ | 10 mM phosphate + 5 mM hexane-sulfonic acid | 3 | 6/4 | 220 | 5 : 54 |
| 3-(3,4-Dichlorophenoxy)-phenethylamine | $CH_3OH$ | 10 mM phosphate + 5 mM hexane-sulfonic acid | 3 | 7/3 | 220 | 6 : 03 |
| Ethylaniline | $CH_3CN$ | 10 mM phosphate | 7 | 1/1 | 235 | 6 : 18 |
| L-Phenylalanine, *tert*-butyl ester | $CH_3CN$ | 10 mM phosphate | 8 | 65/35 | 210 | 4 : 56 |
| *tert*-Butyl 3-amino-3-phenylpropanoate | $CH_3CN$ | 10 mM phosphate | 7 | 65/35 | 215 | 6 : 21 |
| *N*-Hexylpyridinium bromide | $CH_3CN$ | 0·35% triethylamine-phosphate buffer + 0·1% hexanesulfonic acid | 2·5 | 85/15 | 260 | 9 : 05 |
| *N*-Dodecylpyridinium bromide | $CH_3CN$ | 0·35% triethylamine-phosphate buffer + 0·1% hexanesulfonic acid | 2·5 | 8/2 | 260 | 6 : 50 |

exceeding 30°C to leave the aqueous solution. This was transferred to a separating funnel and hydrochloric acid (0·1 M; 3 × 5 ml) was used to rinse the flask. After the addition of saturated sodium chloride (2 ml), the combined aqueous solution was washed with dichloromethane (2 × 25 ml); after adjustment to pH > 12, the compound was extracted with dichloromethane (2 × 25 ml). The organic phase was transferred into a round-bottomed flask together with 10 mM potassium phosphate pH 3 (1 ml), and evaporated at 30°C to leave the aqueous solution. The aqueous phase was made up to 2·0 ml in a volumetric flask with the same buffer solution and the concentration of the test compound was determined by high-pressure liquid chromatography (HPLC).

The analytical procedure was slightly modified for 3-phenoxyphenethylamine. The extracted, filtered and evaporated aqueous solution obtained as above was partitioned with dichloromethane (2 × 25 ml). The organic phase was re-extracted with 0·1 M hydrochloric acid (12·5 ml for roots or 25 ml for shoots). Methanol (30 ml for roots or 40 ml for shoots) was added to the combined aqueous phase and then the mixed solution was extracted with dichloromethane (3 × 25 ml for roots or 3 × 40 ml for shoots) under basic conditions. Subsequent procedures were as above. The analytical procedure was also slightly modified for 3-(3,4-dichlorophenoxy)phenethylamine, L-phenylalanine *tert*-butyl ester, *tert*-butyl 3-amino-3-phenylpropanoate and *N*-dodecylpyridinium bromide as follows. Methanol (25 ml) was added to the extracted, filtered and evaporated aqueous solution as above, and the mixed solution was then partitioned with hexane (2 × 25 ml). The organic phase was discarded, and the aqueous phase was extracted with dichloromethane (2 × 25 ml) under basic conditions (pH > 12). Subsequent procedures were as above.

For *N*-hexylpyridinium bromide, roots and shoots were homogenised with water (2 × 50 ml portions) and the extracts were filtered with suction through glass wool into a round-bottomed flask. The solution was partitioned with hexane (2 × 50 ml) and filtered to break the emulsion. The aqueous phase was separated and evaporated at 60°C to leave about 1 ml of water. This was made up with water to 2·0 ml in a volumetric flask. The concentration of compound in the solution was determined by HPLC.

The recoveries of chemicals from both roots and shoots were more than 80% except for 4-ethylaniline (roots: 69·9%, shoots: 57·6%).

### *2.4.2 High-pressure liquid chromatography*

Concentrations of amines were determined using reverse-phase HPLC under isocratic conditions with UV detection. The HPLC instrument used was a Cecil 1100 Series with a Basic Marathon Autosampler (Spark Holland) and fitted with a Merck column, 25 cm × 4·6 mm ID, with LiChrosorb RP-select B (7 μm). The mobile phase, wavelength of UV detection and retention time for all compounds are shown in Table 2.

### 2.5 Partitioning of compounds onto macerated roots

Freeze-dried macerated roots (0·02 g, equivalent to 0·38 g fresh material) were shaken with 10 mM sodium azide (5 ml) in a centrifuge tube at room temperature for 2 h to remove water-soluble materials that interfered in the HPLC and to sterilise the system. After centrifuging (2000 rev $min^{-1}$, 20 min), the aqueous phase was largely removed to leave an exact weight of roots and water (0·22 g). An aqueous solution of the amine (50 μM, 2·3 ml) buffered as previously to the required pH was added and the mixture shaken gently at room temperature for 2 h. After centrifuging again, the concentration of chemical in both the initial and equilibrium solution was determined by HPLC.

### 2.6 Calculation of results

Uptake of chemicals by roots and efficiency of translocation to shoots were expressed in terms of the Root Concentration Factor (RCF) and Transpiration Stream Concentration Factor (TSCF) respectively, calculated as described by Briggs *et al.*[2,11] Although TSCF was determined indirectly from the accumulation in shoots for a given volume of transpiration, no correction for degradation in the shoot was deemed necessary as the TSCF values so calculated were constant with time over the short uptake periods utilised. All RCF and TSCF results are presented as the mean of the two replicates, each of six plants combined.

## 3 RESULTS

### 3.1 Substituted phenethylamines (Series I)

#### *3.1.1 Uptake by barley roots from solution*

Uptake (as RCF) was constant with time once initial equilibrium was attained, and where possible was measured at 24 and 48 h. However some compounds were rapidly metabolised (in the solution and/or roots) and so, for them, the shorter period of uptake of 6 h had to be utilised. Uptake was only slow to reach equilibrium for the polar 4-mesyl compound, for which RCF at pH 5 to 8 increased approximately four-fold from 6 h to 24 h and continued to increase until at least 72 h. Difficulties were experienced with the two compounds of log $K_{ow} > 3$, for these were phytotoxic (especially 3-(3,4-dichlorophenoxy)phenethylamine) in the high pH solutions and so again short periods of uptake were used; reduction in transpiration rate was a clear indication of phytotoxicity, and this could be seen after 6 h of treatment for these compounds. The RCF values chosen as the most reliable (and used later in Section 4.1.2 for the modelling) are given in Table 3, and plotted with respect to pH and log $K_{ow}$ (Fig. 1).

**TABLE 3**
Uptake by Barley Roots (RCF) and Transport to Shoots (TSCF) of Substituted Phenethylamines (Series I)

| *Substituent* | *Log* $K_{ow}$ | *pH*[a] | *RCF* | *TSCF* |
|---|---|---|---|---|
| 4-Mesyl | −0·04 | 5 | 43·2 | 0·03 |
| | | 6 | 45·1 | 0·04 |
| | | 7 | 38·2 | 0·07 |
| | | 8 | 51·9 | 0·09 |
| 4-Nitro | 1·26 | 5 | 17·3 | 0·17 |
| | | 6 | 14·0 | 0·19 |
| | | 7 | 22·7 | 0·19 |
| | | 8 | 153 | 3·18 |
| 4-Fluoro | 1·63 | 5 | 9·1[b] | 0·21 |
| | | 6 | 9·6[b] | 0·24 |
| | | 7 | 13·3[b] | 0·25 |
| | | 8 | 140[b] | 1·70 |
| 4-Chloro | 2·16 | 5 | 2·1 | 0·18 |
| | | 6 | 3·2 | 0·25 |
| | | 7 | 20·0 | 2·61 |
| | | 8 | 144 | 15·3 |
| 3-Phenoxy | 3·30 | 5 | 2·2[b] | 0·08[b] |
| | | 6 | 4·6 | 0·19 |
| | | 7 | 28·2 | 2·15 |
| | | 8 | 111[b] | 14·0[b] |
| 3-(3,4-Dichloro-phenoxy) | 4·67 | 5 | 17·0[b] | 0·14[b] |
| | | 6 | 37·8[b] | 1·55[b] |
| | | 7 | 59·7[b] | 0·36[b,c] |
| | | 8 | 89·6[b] | 0·24[b,c] |

[a] Solutions of initial pH 5·0 ended at 5·3.
[b] Measured over 6 h; all other values over 24 h.
[c] Strong phytotoxicity observed.

All of the compounds were well taken up by roots, especially from solutions of higher pH. The increase in RCF as the external solution pH was raised from 5·0 to 8·0 was small for the most polar compound (log $K_{ow}$ −0·04), but was much greater as log $K_{ow}$ of compounds increased to 3·30. The RCF values for the most polar

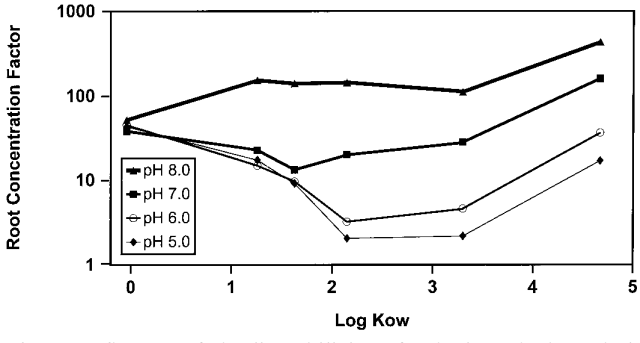



**Fig. 1.** Influence of the lipophilicity of substituted phenethylamines on their uptake by barley roots over 6/24 h from solutions of various pH values.

compound, 4-mesylphenethylamine, at pH 5 to 7 were about 40 to 50, markedly higher than those for the compounds of log $K_{ow}$ 1·26–3·30. At pH 8·0 the RCF increased sharply for all compounds, this increase being most marked with RCF > 100 for the compounds of log $K_{ow}$ > 2 for which some increase could also be seen at solution pH of 6 to 7. The most lipophilic compound was strongly accumulated from all solutions (pH 5·0 to 8·0), although phytotoxicity makes these data uncertain.

*3.1.2 Translocation to shoots*

Transport from roots to shoots (selected values in Table 3, Fig. 2) showed the same dependence on pH as did uptake by roots, and the TSCF values likewise rapidly reached equilibrium for all but the most polar compound. TSCF values were rather similar for all the substituted phenethylamines at pH 5, but increased sharply at pH 7 to 8. The most marked increases in TSCF occurred for the compounds of log $K_{ow}$ > 2, for which the increase in TSCF was well noticeable at pH 7 and discernible even at pH 6. TSCF values were greater than unity for all the compounds of intermediate lipophilicity at pH 8 and, for some, at pH 7; within these, the 4-Cl and 4-PhO compounds were remarkably well translocated with TSCF ~ 10 at pH 8.

*3.1.3 Partition onto macerated roots*

All the bases were measurably partitioned onto root solids, with the extent of partitioning increasing slightly as solution pH was increased (Fig. 3a). The $RCF_{macerated}$ values increased markedly with lipophilicity (Fig. 3b), following linear free-energy relationships with the most lipophilic compound being strongly partitioned ($RCF_{macerated}$ > 80, at pH 8).

## 3.2 Bases of various pKa (Series II)

*3.2.1 Uptake by barley roots from solution*

4-Ethylaniline and the *tert*-butyl ester of L-phenylalanine were rapidly metabolised and so a short period of uptake (6 h) was utilised. No phytotoxicity

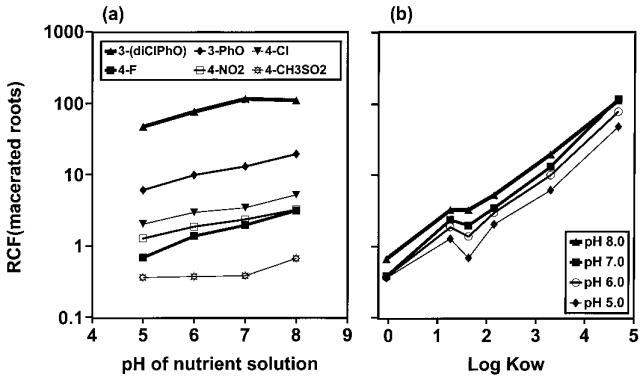



**Fig. 3.** Partitioning of substituted phenethylamines onto macerated root solids from solution: (a) effect of solution pH; (b) relationship to log $K_{ow}$.

was observed with these compounds, and all were well taken up by roots especially from the solutions of higher pH (Table 4, selected examples for 6 or 24 h). The increase in RCF as the external solution pH (Fig. 4) was raised from 4 to 8 was small for the weak base 4-ethylaniline (pKa 5·03), but was much greater as basicity of the compound increased (pKa to 9·80). The RCF values for 4-ethylaniline at pH 5 and 6 were modest though higher than those for stronger bases. However, unlike 4-ethylaniline, the RCF increased sharply at pH 6 and 7 for L-phenylalanine *tert*-butyl ester (pKa 7·44), at pH 7 and 8 for *tert*-butyl 3-amino-3-phenylpropanoate (pKa 8·03) and at pH 8 for 4-chlorophenethylamine (pKa 9·80). The most marked increase in RCF (approximately 5- to 6-fold from pH 7 to 8) occurred for the two strongest bases, *tert*-butyl 3-amino-3-phenylpropanoate and 4-chlorophenethylamine, which were strongly taken up by roots at pH 8, reaching RCF values of 103 and 144, respectively.

*3.2.2 Translocation to shoots*

Transport from roots to shoots showed the same dependence on pH (Table 4) as did uptake by roots, with TSCF values being rather similar for all the bases at pH 4 but increasing sharply at higher pH for the three stronger bases (Fig. 5). TSCF values were greater than

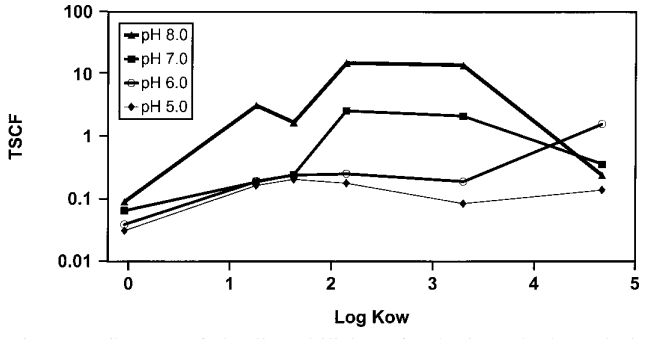



**Fig. 2.** Influence of the lipophilicity of substituted phenethylamines on their translocation to barley shoots over 6/24 h following uptake from solutions of various pH values.

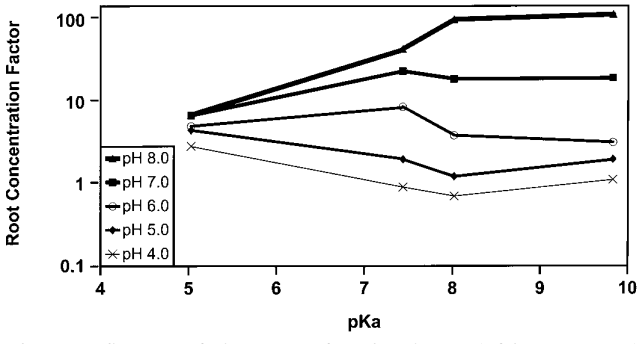



**Fig. 4.** Influence of the pKa of amine bases (of log $K_{ow}$ ~ 2) on their uptake by barley roots over 6/24 h from solutions of various pH values.

**TABLE 4**
Uptake by Barley Roots (RCF) and Transport to Shoots (TSCF) of Bases of Various pKa (Series II)

| *Compound* | *pH of external solution*[a] | $RCF_{macerated}$ | *RCF* | *TSCF* |
|---|---|---|---|---|
| 4-Ethylaniline | 4 | 3·2 | 2·9 | 0·10 |
| | 5 | 3·8 | 4·6 | 0·09 |
| | 6 | 5·2 | 5·1 | 0·11 |
| | 7 | 7·9 | 7·0 | 0·21 |
| | 8 | 8·7 | 7·1 | 0·17 |
| L-Phenylalanine, | 4 | 1·7 | 0·9 | 0·17 |
| *tert*-butyl ester | 5 | 2·8 | 2·0 | 0·46 |
| | 6 | 4·0 | 8·8 | 2·58 |
| | 7 | —[b] | 24·1 | 5·85 |
| | 8 | 5·6 | 45·0 | 14·6 |
| *tert*-Butyl 3-amino-3- | 4 | 0·6 | 0·7 | 0·27 |
| phenylpropanoate | 5 | 0·8 | 1·2 | 0·37 |
| | 6 | 1·2 | 4·0 | 1·20 |
| | 7 | 1·7 | 19·4 | 5·43 |
| | 8 | 2·1 | 103 | 23·2 |
| 4-Chloro- | 4 | 1·7 | 1·1 | 0·13 |
| phenethylamine | 5 | 2·1 | 2·1[c] | 0·18[c] |
| | 6 | 3·0 | 3·2[c] | 0·25[c] |
| | 7 | 3·5 | 20[c] | 2·61[c] |
| | 8 | 5·3 | 144[c] | 15·3[c] |

[a] Initial pH values of 4·0 and 5·0 drifted to 4·6 and 5·3 respectively by the end of the experiment.
[b] Rapid degradation precluded measurement.
[c] These values were measured at 24 h; all other RCF and TSCF values were measured at 6 h.

unity for compounds of intermediate basicity at pH 7 and 8, though for the strongest base only at pH 8. *tert*-Butyl 3-amino-3-phenylpropanoate was extremely effectively translocated to shoots at pH 8, with a TSCF of 23.

### *3.2.3 Partition onto macerated roots*

Partitioning onto macerated roots increased slightly as solution pH was increased (Table 4), as was also observed for the strong bases of series I, but $RCF_{macerated}$ values were rather low, not exceeding 8·7.

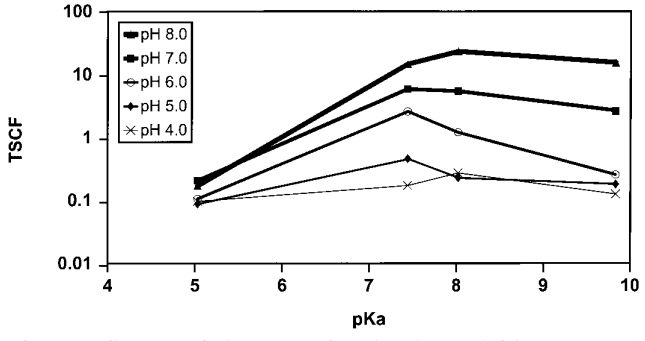



**Fig. 5.** Influence of the pKa of amine bases (of log $K_{ow} \sim 2$) on their translocation to barley shoots over 6/24 h following uptake from solutions of various pH values.

## 3.3 *N*-Alkylpyridinium salts (Series III)

### *3.3.1 Uptake by barley roots from solution*

The RCF values (Fig. 6a) were constant with pH for both pyridinium salts. Uptake was slow to reach equilibrium for the very polar *N*-hexylpyridinium bromide, for which RCF increased greatly beyond 6 h; it continued to increase (approximately two-fold) from 48 h to 72 h, by which time the very high RCF value of 203 (at

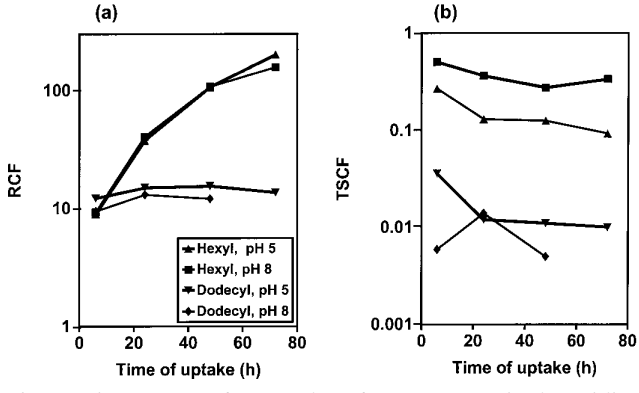



**Fig. 6.** Time course for uptake of two quaternised pyridine cations by barley roots from solutions of various pH values and subsequent translocation to shoots.

pH 5) was obtained. In contrast, uptake of *N*-dodecylpyridinium chloride rapidly reached equilibrium, giving a smaller accumulation of RCF ~ 15.

*3.3.2 Translocation to shoots*

Transport from roots to shoots was constant with time after 24 h, although the TSCF values for 6 h were generally slightly higher than those for the longer periods (Fig. 6b). The TSCF values were higher for the *N*-hexylpyridinium cation, at pH 8 than at pH 5; little difference with pH was discernible for the *N*-dodecylpyridinium cation, though phytotoxicity at pH 8 beyond 48 h precluded measurements at 72 h. The *N*-dodecylpyridinium cation, with TSCF values at pH 5 and 8 of 0·012 and 0·13 respectively, was much less efficiently translocated to shoots than the *N*-hexylpyridinium.

*3.3.3 Partition onto macerated roots*

Partitioning onto macerated roots was constant with pH, with *N*-dodecylpyridinium chloride being strongly partitioned ($RCF_{macerated}$ 50·4 and 60·9 at pH 5 and 8 respectively), whereas *N*-hexylpyridinium bromide was much less so ($RCF_{macerated} < 0{\cdot}6$).

## 4 DISCUSSION

### 4.1 Uptake by roots

*4.1.1 Uptake processes*

Three processes contribute to the uptake of bases by roots. These are the accumulation of cations according to the Nernst equation, accumulation by ion trapping if the plant compartments are of a lower pH than the external solution and partitioning onto root solids. These will be considered in turn.

*4.1.1.1 Accumulation of cations inside root cells by the Nernst effect.* The Nernst effect is the process whereby the negative charge on the plasmalemma membrane bounding the cell leads to accumulation of cations given by:

$$E(\text{mV}) = 59 \log \frac{\text{concentration (inside cell)}}{\text{concentration (external soln)}} \quad (1)$$

For a monocation, such accumulation is predicted to be about 100-fold for a typical membrane potential of − 120 mV. However, the membrane potential can have different values according to plant species; even within a species such as barley it is dependent, amongst other factors, on the $K^+$ status of the bathing medium, varying from −80 mV at high $K^+$ to around −200 mV in distilled water.[13,14]

The permanent cation *N*-hexylpyridinium bromide was strongly accumulated, albeit slowly, by roots. 4-Mesylphenethylamine, the most polar of the strong bases (Series I), was also well accumulated from solution even at pH 5 (Fig. 1); the values of RCF ~ 50 at 24 h are much larger than the estimated value of 0·85 for a non-ionised compound having the same lipophilicity as the free base. These compounds are thus accumulated as the cation by the cell as a consequence of the negative charge on the membrane; the very high RCF values (~200) attained after 72 h cannot be explained by a Nernst potential of only −80 mV, and a possible explanation is that these compounds are causing this potential to fall to less than −120 mV.

The more lipophilic of our strong bases can also be accumulated as their cations, but now outwards leakage of the non-ionised species, despite their concentrations in the cytoplasm being only 1% of those of their cations, can undermine the Nernst effect. As log $K_{ow}$ for the phenethylamines was increased to 2·16, the non-ionised form permeated membranes more efficiently, as shown both by the high TSCF values and the low RCF values at pH 5, indicating that the charge effects were much smaller. Our buffer solutions at pH 4 to 7 contained high $K^+$ (10–14 mM) and for tests at pH 8, for compounds where the Nernst effect was important, $K^+$ was added to 10 mM. It was important for 4-mesylphenethylamine and *N*-alkylpyridinium salts to maintain the concentration of $K^+$ in external solution, as the Nernst effect predominated for these compounds during root uptake; tests with the other compounds indicated that RCF values were not decreased by the addition of $K^+$ to the solutions (data not shown). Uptake of *N*-dodecylpyridinium chloride rapidly reached equilibrium, in contrast to that of *N*-hexylpyridinium bromide, though accumulation of the former was less; the longer alkyl chain of the *N*-dodecylpyridinium cation is thought to enhance its permeation through membranes (possibly through formation of an ion pair) and perhaps to shield the charge on the cation so lessening its accumulation.

*4.1.1.2 Ion trapping.* A second uptake process is the ion-trap mechanism whereby the different permeation rates of the non-ionised form and its respective cation allow bases to be accumulated in those plant compartments of lower pH. Entry of the bases into root tissue is primarily by diffusion of the non-ionised species, which permeate membranes much more rapidly than their respective cations. Once inside the cell, protonation occurs and the cations are unable to diffuse out very readily, thus leading to their accumulation when the pH of the external solution is more than that of the plant compartments (cytoplasm pH 7·5, vacuole pH 5·5). Diffusion occurs until equilibrium is established between the concentration of the free base in the cell and that in the external solution. Therefore, both dissociation in the plant compartments and the pH of the external solution influence the extent of ion trapping, with sharply increasing uptake as the pH of the external solution is increased.

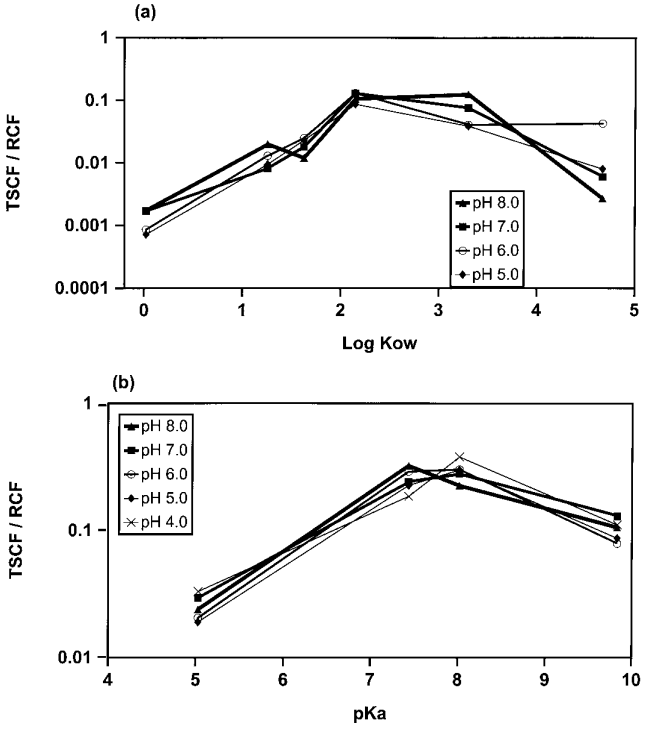



**Fig. 7.** Efficiency of translocation to barley shoots relative to uptake by roots from solutions of various pH values: (a) influence of the lipophilicity of substituted phenethylamines; (b) influence of the pKa of amine bases (of log $K_{ow} \sim 2$).

Within our series of substituted phenethylamines in which these factors were essentially constant, the extent of accumulation also depended on the relative permeation rate through membranes of the free base to its protonated cationic species ($P_B/P_{BH+}$). This ratio was a function of the lipophilicity of the base. At solution pH 6, uptake by ion trapping can only be very small and at pH 5 ion trapping now excludes compound from the roots. The high uptake of the polar 4-mesylphenethylamine (RCF $\sim$ 45 at pH $\leqslant$ 7) and its failure to increase markedly at pH 8 indicate both a low $P_B$ and a low permeability ratio $P_B/P_{BH+}$ insufficient respectively to undermine the Nernst effect or to give appreciable ion trapping. As lipophilicity was increased *via* the 4-$NO_2$ and 4-F to the 4-Cl compound, RCF at pH 5 fell sharply and at pH 8 increased greatly, indicating an increasingly important role for ion trapping, due to increasing $P_B/P_{BH+}$ ratios.

Compounds of intermediate lipophilicity (Series II) were accumulated in roots mainly by ion-trapping (Fig. 4), the Nernst effect being insignificant. For the strongest base, 4-chlorophenethylamine (pKa 9·80), the concentration of non-ionised species is very low at pH 4 to 7, so that permeation is here dominated by movement of the ionised species and accumulation by ion trapping is weak; effective ion-trapping only begins at pH 8 as the pKa is approached. In contrast, L-phenylalanine *tert*-butyl ester (pKa 7·44) would be about 50% protonated in the cytoplasm (pH 7·5) increasing to 99% in the vacuole (pH 5·5). This compound, and likewise *tert*-butyl 3-amino-3-phenylpropanoate (pKa 8·03), can thus be effectively ion-trapped at pH values down to 7 to even 6. The small RCF values for the weakest base, 4-ethylaniline (pKa 5·03), were due to its lack of protonation in the plant compartments.

*4.1.1.3 Partitioning onto root solids.* A third process is partitioning onto root solids, measured using freeze-dried macerated roots and important only for the more lipophilic bases. Such partitioning of bases increased moderately as the pH of the solution increased (Fig. 3a, Table 4), whereas partitioning of the permanent cations was largely independent of the solution pH. The cations were thus less strongly partitioned than the free bases, partitioning of which appeared to be the dominant process even at pH values well below the pKa as the measured $RCF_{macerated}$ values were close to those estimated using the log $K_{ow}$ values of the free bases.[2]

The $RCF_{macerated}$ values for 3-(3,4-dichlorophenoxy)-phenethylamine, 3-phenoxyphenethylamine and *N*-dodecylpyridinium chloride were higher than the respective RCF values obtained for roots of intact plants, indicating that bases are partitioned onto root solids according to the different concentration in each plant compartment.

*4.1.2 Prediction of accumulation in roots*

The magnitude of accumulation by the Nernst effect and the ion-trap mechanism can be predicted using the equation:[6,7]

$$\frac{[B]_i + [BH^+]_i}{[B]_o + [BH^+]_o} = \frac{(1 + 10^{pKa - pHi})[P_B/P_{BH+} + \{(FE/RT)/(1 - e^{FE/RT})\} \cdot 10^{pKa - pHo}]}{(1 + 10^{pKa - pHo})[P_B/P_{BH+} + \{(FE/RT)/(1 - e^{FE/RT})\}10^{pKa - pHi} \cdot e^{FE/RT}]} \quad (2)$$

where pHi and pHo are respectively the pH inside and outside the membrane, F is the Faraday constant (96 479 coulombs $mol^{-1}$), E is the charge on the membrane (mV), R is the universal gas constant (8·31 joules $K^{-1}$ $mol^{-1}$), T is the absolute temperature (K) and $P_B$ and $P_{BH+}$ are the permeation rates through the membrane of the free base and protonated forms of the amines respectively. The vacuole and cytoplasm were assumed to occupy 90% and 10% respectively of the cell volume, and their respective pH values were taken to be 5·5 and 7·0. The plasmalemma potential was taken to be $-80$ mV, as the solution bathing the roots was high in potassium.[13] The tonoplast potential was assumed to be negligible. Therefore, eqn (2) can be simplified for the estimation of the accumulation in vacuole by ignoring the Nernst effect:

$$\frac{[B]_i + [BH^+]_i}{[B]_o + [BH^+]_o} = \frac{(1 + 10^{pKa - pHi})P_B/P_{BH+}}{(1 + 10^{pKa - pHo})P_B/P_{BH+}} \quad (3)$$

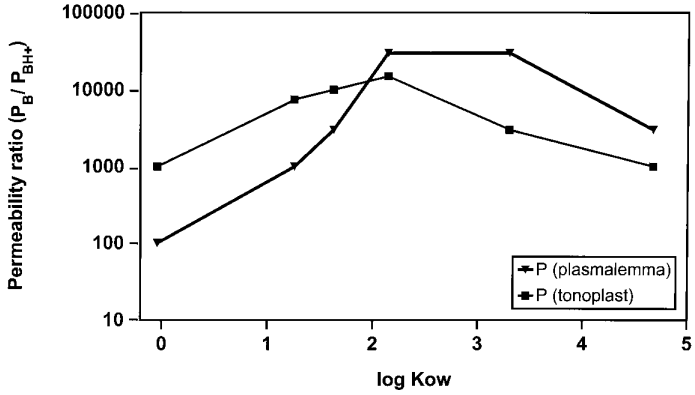



**Fig. 8.** Influence of log $K_{ow}$ on the permeability ratios, $P_B/P_{BH+}$, in barley roots for the substituted phenethylamines.

Our approach to prediction was based on the observation that the efficiency of transport of the bases from roots to shoots, assessed using TSCF/RCF ratios derived from the data in Tables 3 and 4, was independent of external pH and showed clear relationships with log $K_{ow}$ and pKa (Fig. 7). The optimum log $K_{ow}$ for such transport was 2 to 3, which is the same as that for non-ionised compounds in terms of TSCF. The optimum pKa was 7·5 to 8 which is the same as the pH of cytoplasm and the pKa of the morpholine fungicides.[11] However, use of the TSCF/RCF ratio is itself an oversimplification, in that it comprises the different concentrations in the cytoplasm and vacuole, together with partitioning onto the root solids. Therefore, the

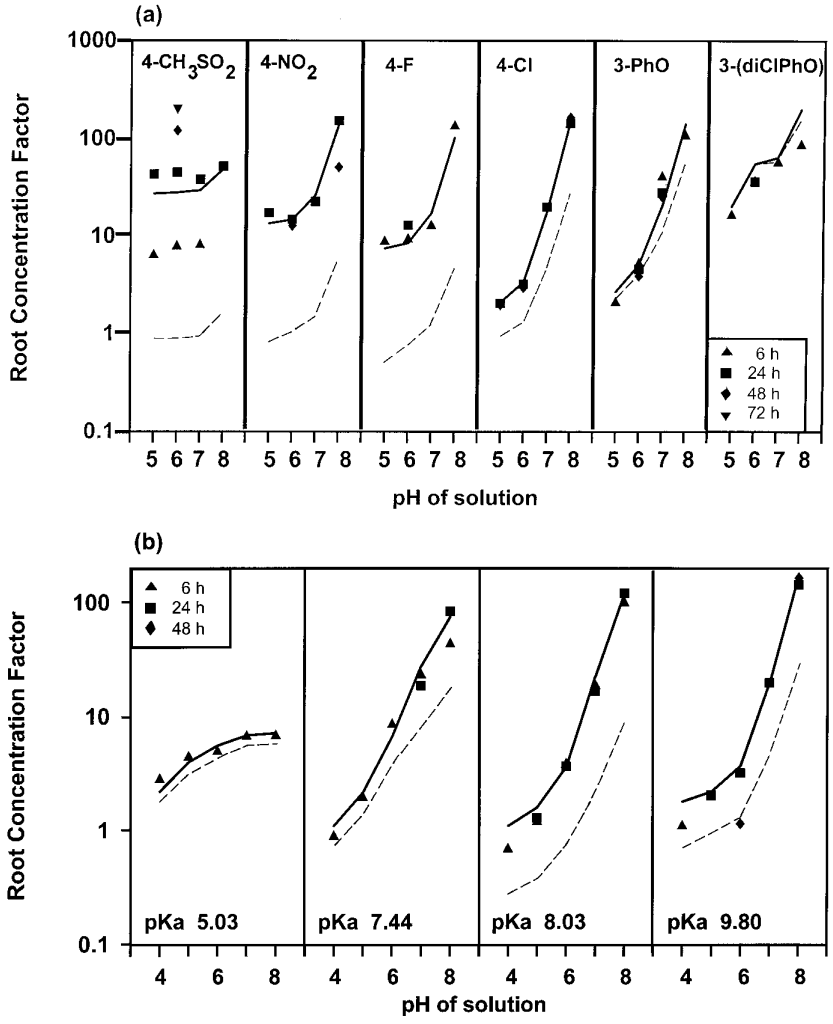



**Fig. 9.** Modelling of uptake by barley roots from solutions of various pH values: (a) substituted phenethylamines in order of increasing lipophilicity; (b) amine bases of log $K_{ow} \sim 2$ but different pKa values. The dashed lines indicate the contribution of partitioning.

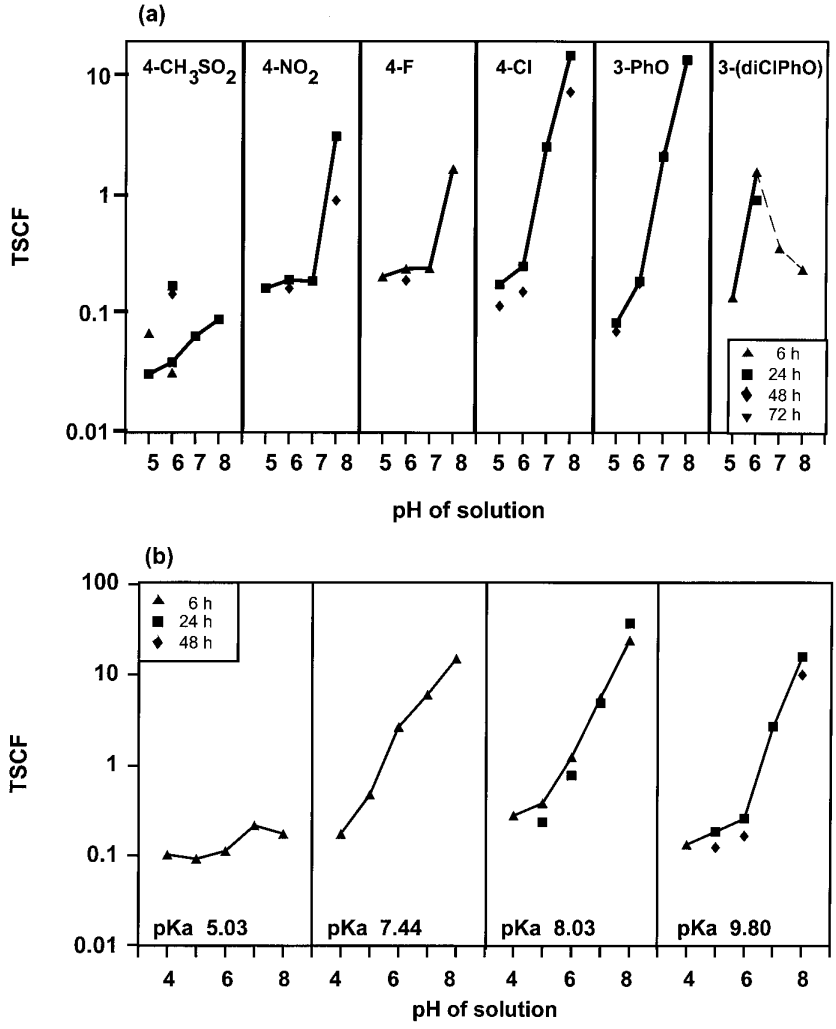



**Fig. 10.** Translocation to shoots following uptake by barley roots from solutions of various pKa values: (a) substituted phenethylamines (the most lipophilic was phytotoxic above pH 7); (b) amine bases of log $K_{ow} \sim 2$ but different pKa values.

ratio of 'TSCF/cytoplasm concentration' is a better indicator of permeation from the cytoplasm to the xylem.

The ratios of the permeabilities of both the plasmalemma and tonoplast to the free and protonated forms of the bases were thus optimised according to the following criteria. The first criterion was the minimum variance of the ratio of measured value of TSCF to the concentration of the base in cytoplasm, which can be calculated according to eqn (3) and which approach avoids the complications of the contributions of partitioning to RCF. The second criterion was the best fit between measured RCF values and the calculated RCF values. The optimum lipophilicity for permeation ratios differed between the plasmalemma and tonoplast (Fig. 8), being log $K_{ow}$ 2 to 3 for the former and log $K_{ow} \sim 2$ for the tonoplast.

For the substituted phenethylamines of intermediate lipophilicity, the cytoplasm concentrations in root cells were estimated to be low at external solution pH 5, being from 0·014 to 0·24 of that outside, but increased sharply at pH 8 to $> 3$; concentrations in the vacuole were estimated to be 32 to 44 times those in the cytoplasm, indicating the important role of the vacuole in accumulating such bases.

Thirdly, the magnitude of the partitioning onto root solids was estimated by considering the roots as composed of three compartments. Firstly, there is the outside of the cell wall onto which bases are partitioned at the concentration and pH of the external solution. Secondly, partitioning occurs in the cell onto the inside of the cell wall at the concentration and pH of the cytoplasm. The third compartment is the vascular bundle where bases were assumed to be partitioned onto xylem solids at the concentration (i.e. TSCF) and pH of the xylem. It was assumed that each partitioning compartment contributes 33% of the total partitioning potential, and, though arbitrary, this assumption led to a good fit to the RCF data.

The predicted RCF values encompassing all these processes are compared to the complete data set for the Series I and II bases (Fig. 9); for 4-mesylphenethylamine for which the RCF increased markedly with time, the RCF values at 24 h were fitted. Also shown is the contribution of partitioning, appreciable only for 4-

ethylaniline and the more lipophilic phenethylamines. The fit was generally excellent across the pH range; this leads us to believe that uptake by roots of bases can be attributed to recognised physicochemical processes.

### 4.2 Transport to shoots

Movement to shoots (Fig. 10) was controlled by the same physicochemical processes as uptake by roots, and these parallels can be seen by comparison with the RCF values (Fig. 9). Exceptions were 4-mesylphenethylamine for which the Nernst effect leads to strong uptake by root cells but poor long-distance transport, and the most lipophilic phenethylamine for which phytotoxicity limited transport at pH 7 and 8.

The parabolic relationships between log $K_{ow}$ and the ratio of TSCF/cytoplasm concentration (Fig. 11a) are similar to those between log $K_{ow}$ and TSCF/RCF (Fig. 7a), again being independent of solution pH, and the same was also true for the relationship of these ratios to pKa (Figs 11b and 7b). The optimum log $K_{ow}$ for movement from root cytoplasm to xylem was also log $K_{ow}$ 2 to 3, and the optimum pKa 7·5 to 8.

The ratios of TSCF/cytoplasm concentration for the phenethylamines of intermediate lipophilicity and the bases of pKa 7·4 to 8 were greater than unity, and indeed greater than 10-fold for several compounds. This indicates that the process of transport from cytoplasm to xylem is due not only to diffusion but also the ion-trap process between these compartments of different pH values. However, this ion-trap process appeared not to attain equilibrium, for these ratios, though high, were still substantially less than the equilibrium ion-trapping from cytoplasm to xylem sap estimated using eqn. (2).

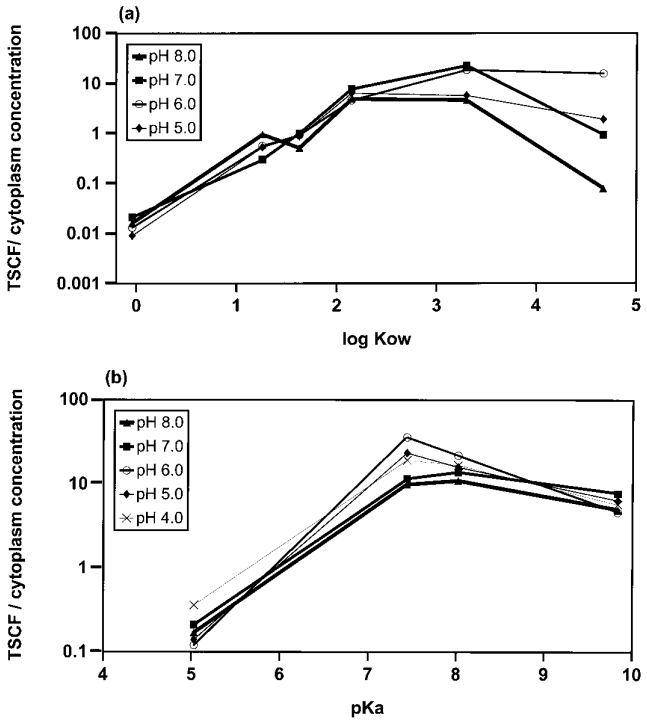



**Fig. 11.** Efficiency of transport from cytoplasm to xylem sap in barley roots following uptake from solutions of various pH values: (a) substituted phenethylamines; (b) bases of log $K_{ow} \sim 2$ but different pKa values.

In the substituted phenethylamines, the measured ratios for the 4-mesyl and 3-phenoxy compounds were only about 5% of the equilibrium values, but for the three compounds of intermediate lipophilicity they approached 20%. However, this ratio was between 25 and 30% for the compounds of log $K_{ow} \sim 2$ and intermediate basicity (pKa 7·44 and 8·03). This indicates that the approach to equilibrium in the ion-trapping between cytoplasm and xylem is primarily due to the proportion of non-ionised species in the cytoplasm, and so the strong bases of intermediate lipophilicity were not able to make full use of their large permeation ratios due to the rapid flux of water in the xylem limiting the time available for such uptake.

## 5 CONCLUSIONS

The systemicity of compounds with similar properties to the amine fungicides is explicable by physicochemical processes. When the bases were applied in solution to the roots at pH 4 to 6, accumulation by barley roots and transport to shoots was generally poor, but at pH 7 and 8 became increasingly efficient. These processes were particularly effective for compounds of intermediate lipophilicity (log $K_{ow}$ 2 to 3) and intermediate pKa (7·5 to 8·5). A polar quaternised pyridine and the polar 4-mesylphenethylamine were strongly accumulated in roots as the cations by the Nernst effect, but their subsequent translocation was poor. As previously noted with the lipophilic morpholine fungicides, such excellent transport *via* xylem due to ion-trapping at the low pH of xylem sap could lead to opportunities to confer improved systemicity on non-ionised classes of pesticides by introducing an appropriate amine base function.

## 6 ACKNOWLEDGEMENTS

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